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TITLE: Nematode-extracted anticoagulant protein

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CLAIMS:

We claim:
1. An isolated protein having Factor VIIa/TF inhibitory activity and having one or more Nematode-extracted Anticoagulant Protein domains ("NAP domains"), wherein each NAP domain includes the sequence;
Cys-A1-Cys-A2-Cys-A3-Cys-A4-Cys-A5-Cys-A6-Cys-A7-Cys-A8-Cys-A9-Cys-A10, wherein
(a) A1 is an amino acid sequence of 7 to 8 amino acid residues
(b) A2 is an amino acid sequence;
(c) A3 is an amino acid sequence of 3 amino acid residues;
(d) A4 is an amino acid sequence;
(e) A5 is an amino acid sequence of 3 to 4 amino acid residues;
(f) A6 is an amino acid sequence;
(g) A7 is an amino acid residue;
(h) A8 is an amino acid sequence of 11 to 12 amino acid residues;
(i) A9 is an amino acid sequence of 5 to 7 amino acid residues; and
(j) A10 is an amino acid sequence;
wherein each of A2, A4, A6 and A10 has an independently selected number of independently selected amino acid residues and each sequence is selected such that each NAP domain has in total less than about 120 amino acid residues and wherein said isolated protein is derived from a hematophagous nematode species.
2. The protein of claim 1, wherein A3 has the sequence Asp-A3.sub.a -A3.sub.b, wherein A3.sub.a and A3.sub.b are independently selected amino acid residues.
3. The protein of claim 1, wherein A3 is Asp-Lys-Lys.
4. The protein of claim 1, wherein A4 is an amino acid sequence having a net anionic charge.
5. The protein of claim 1, wherein A5 has the sequence A5.sub.a -A5.sub.b -A5.sub.c -A5.sub.d [SEQ. ID. NO. 45], wherein A5.sub.a through A5.sub.d are independently selected amino acid residues.
6. The protein of claim 5, wherein A5.sub.a is Leu and A5.sub.c is Arg.
7. The protein of claim 1, wherein A7 is selected from the group consisting of Val and Ile.

8. The protein of claim 1, wherein A7 is Val.
9. The protein of claim 1, wherein A8 is [illegible] -A8.sub.b -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f -A8.sub.g - [illegible], wherein
(a) A8.sub.a is the first amino acid residue in A8,
(b) at least one of A8.sub.a and A8.sub.b is selected from the group consisting of Glu or Asp, and
(c) A8.sub.c through A8.sub.g are independently selected amino acid residues.
10. The protein of claim 9, wherein
(a) A8.sub.a is Glu or Asp,
(b) A8.sub.b is an independently selected amino acid residue,
(c) A8.sub.c is Gly,
(d) A8.sub.d is selected from the group consisting of Phe, Tyr, and Leu,
(e) A8.sub.e is Tyr,
(f) A8.sub.f is Arg, and
(g) A8.sub.g is selected from Asp and Asn.
11. The protein of claim 10, wherein -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f -A8.sub.g - is Gly-Phe-Tyr-Arg-Asn [SEQ. ID. NO. 70].
12. The protein of claim 9, wherein
(a) A8.sub.a is an independently selected amino acid residue,
(b) A8.sub.b is Glu or Asp,
(c) A8.sub.c is Gly,
(d) A8.sub.d is selected from the group consisting of Phe, Tyr, and Leu,
(e) A8.sub.e is Tyr,
(f) A8.sub.f is Arg, and
(g) A8.sub.g is selected from Asp and Asn.
13. The protein of claim 12, wherein -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f -A8.sub.g - is -Gly-Phe-Tyr-Arg-Asn [SEQ. ID. NO. 70].
14. The protein of claim 1, wherein said nematode species is selected from the group consisting of Ancylostoma caninum, Ancylostoma ceylanicum, Ancylostoma duodenale, Necator americanus, and Heligomosomoides polygyrus.
15. The protein of claim 1, wherein
(a) A3 is has the sequence Asp-A3.sub.a -A3.sub.b, wherein A3.sub.a and A3.sub.b are independently selected amino acid residues;
(b) A4 is an amino acid sequence having a net anionic charge;
(c) A5 has the sequence A5.sub.a -A5.sub.b -A5.sub.c -A5.sub.d [SEQ. ID. NO. 85], wherein A5.sub.a through A5.sub.d are independently selected amino acid residues, and

(d) A7 is selected from the group consisting of Val and Ile.
16. The protein of claim 15 having a NAP domain of AcaNAPc2 (SEQ. ID. NO. 59).
17. The protein of claim 15, wherein said nematode species is selected from the group consisting of Ancylostoma caninum, Ancylostoma ceylanicum, Ancylostoma duodenale, Necator americanus, and Heligomosomoides polygyrus.
18. The protein of claim 1, wherein
(a) A3 is Asp-Lys-Lys;
(b) A4 is an amino acid sequence having a net anionic charge;
(c) A5 has the sequence A5.sub.a -A5.sub.b -A5.sub.c -A5.sub.d, wherein A5.sub.a is Leu, A5.sub.c is Arg, and A5.sub.b and A5.sub.d are independently selected amino acid residues [SEQ. ID. NO. 357],
(d) A7 is Val; and
(e) A8 includes an amino acid sequence A8.sub.a -A8.sub.b -Gly -Phe-Tyr-Arg-Asn [SEQ. ID. NO. 79], wherein at least one of A8.sub.a and A8.sub.b is Glu or Asp.
19. The protein of claim 18 having a NAP domain of AcaNAPc2 (SEQ. ID. NO. 59).
20. The protein of claim 18, wherein said nematode species is selected from the group consisting of Ancylostoma caninum, Ancylostoma ceylanicum, Ancylostoma duodenale, Necator americanus, and Heligomosomoides polygyrus.
21. An isolated protein having Factor VIIa/TF inhibitory activity having a NAP domain with an amino acid sequence of AcaNAPc2 (SEQ. ID. NO. 59).
22. A pharmaceutical composition comprising the protein of claim 1.
23. A pharmaceutical composition comprising the protein of claim 15.
24. A pharmaceutical composition comprising the protein of claim 18.
25. A pharmaceutical composition comprising an AcaNAPc2 protein [SEQ. ID. NO. 59].
26. A method of inhibiting blood coagulation comprising administering a protein of claim 1 with a pharmaceutically acceptable carrier.
27. A method of inhibiting blood coagulation comprising administering a protein of claim 15 with a pharmaceutically acceptable carrier.
28. A method of inhibiting blood coagulation comprising administering a protein of claim 18 with a pharmaceutically acceptable carrier.
29. A method of inhibiting blood coagulation comprising administering an AcaNAPc2

protein (SEQ. ID. NO. 59 .
30. A protein of claim 1, wherein said protein has two NAP domains.
31. A protein of claim 15, wherein said protein has two NAP domains.
32. A protein of claim 1*, wherein said protein has two NAP domains.
33. An isolated protein having anticoagulant activity, wherein said protein specifically inhibits the catalytic activity of the fVIIa:TF complex in the presence of fXa or catalytically inactive fXa derivative and not in the absence of fXa or catalytically inactive fXa derivative and does not specifically inhibit the activity of fVIIa in the absence of TF and does not specifically inhibit prothrombinase and wherein said protein is derived from a hematophagous nematode species.
34. An isolated protein having an amino acid sequence of AcaNAP5 (SEQ. ID. NO. 59 , wherein said protein specifically inhibits the catalytic activity of the fVIIa:TF complex in the presence of fXa or catalytically inactive fXa derivative, and does not specifically inhibit the activity of FVIIA in the absence of TF and does not specifically inhibit prothrombinase.
35. An isolated protein having an amino acid sequence of AcaNAP*2 (SEQ. ID. NO. 5* .
36. A protein having an amino acid sequence of AcaNAP52/proline.
37. A method of treating a pathologic condition characterized by abnormal thrombosis by preventing or decreasing said abnormal thrombosis, which comprises administering a protein of claim 1.
38. A method according to claim 37 wherein said pathologic condition is disseminated intravascular coagulopathy.
39. A method of treating a pathologic condition characterized by abnormal thrombosis by preventing or decreasing said abnormal thrombosis, which comprises administering a protein of claim 15.
40. A method according to claim 39 wherein said pathologic condition is disseminated intravascular coagulopathy.
41. A method of treating a pathologic condition characterized by abnormal thrombosis by preventing or decreasing said abnormal thrombosis, which comprises administering a protein of claim 18.
42. A method according to claim 41 wherein said pathologic condition is disseminated intravascular coagulopathy.
43. A method of treating a pathologic condition characterized by abnormal thrombosis by preventing or decreasing said abnormal thrombosis, which comprises administering a protein of claim 21.
44. A method according to claim 43 wherein said pathologic condition is disseminated intravascular coagulopathy.
45. A method of treating a pathologic condition characterized by abnormal thrombosis by preventing or decreasing said abnormal thrombosis, which comprises administering a protein of claim 33.
46. A method according to claim 45 wherein said pathologic condition is disseminated intravascular coagulopathy.
47. A method of treating a pathologic condition characterized by abnormal thrombosis by preventing or decreasing said abnormal thrombosis, which comprises administering a protein of claim 35.
48. A method according to claim 47 wherein said pathologic condition is disseminated intravascular coagulopathy.
49. A method of treating a pathologic condition characterized by abnormal thrombosis by preventing or decreasing said abnormal thrombosis, which comprises administering a protein of claim 36.
50. A method according to claim 49 wherein said pathologic condition is disseminated intravascular coagulopathy.